ライソゾーム・カテプシンLの生合成，細胞内プロセシングおよびその活性化

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INTRACELLULAR PROCESSING AND ACTIVATION OF LYSOSOMAL CATHEPSIN L

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ABSTRACT The biosynthesis and intracellular processing of lysosomal cysteine proteases cathepsin L was studied using *in vitro* translation system and *in vivo* pulse-chase analysis with [^{35}S] methiohine and [^{32}P] phosphate in cultured rat hepatocytes. RNA prepared from membrane-bound but not free polysomes directed the synthesis of a primary translation product of an immunoprecipitable 37.5-kDa cathepsin L *in vitro*. The 37.5-kDa form was converted to the 39-kDa form when translated in the presence of dog pancreas microsomes. During pulse-chase experiments in cultured rat hepatocytes, cathepsin L was first synthesized as a 39-kDa protein and was subsequently prossed into the mature forms with 30-kDa and 23-kDa in the cell. However, it is notable that considerable amounts of the proenzyme was found to be secreted into the culture medium without further proteolytic processing. The precursor and mature enzymes were N-glycosylated with high-mannose type oligosaccharides, and the proenzyme molecule contained phosphorylated oligosaccharides. The proform of cathepsin L was also found in the rat liver microsomal contents by immunoblot analysis. The proenzyme was partially purified by Con A-Sepharose chromatography. The Con A-adsorbed fractions containing the proenzyme showed no appreciable cathepsin L activity. When this fraction was incubated at pH 3.0, the enzyme activity markedly increased ; cathepsin Lactivity after 36 h incubation was 210-times that of the control. Immunoblot analysis demonstrated that after 36 h incubation the proenxyme disappeared and the mature enzyme increased. The marked increase of enzymatic activity and the conversion of the proenzyme to the mature from were completely

bloced with pepstatin. Therefore, procathepsin L seems to be synthesized as an enzymatically inactive form in the endopiasinic reticulum and may be conuerted to an active form by aspartic protease.

抄録　ライソゾーム酵素であるカテプシンLの生合成と細胞内プロセシングを，[^{35}S]メチオニンや[^{32}P]リン酸を標識化合物として用い，*in vitro* 翻訳実験ならびに初代培養ラット肝細胞におけるパルスーチェィス実験を行って調べた。カテプシンLは膜結合ポリゾームのRNAによっては37.5 kDaの一次翻訳生成物が生合成されたが，フリーポリゾームのRNAによっては生合成されなかった。この3.75 kDaの蛋白はマイクロゾームが存在すると39 kDa蛋白へ転換した。培養細胞内では，カテプシンLは最初，39 kDaの蛋白として得られ，時間がたつと成熟型酵素である30 kDaあるいは23 kDa蛋白へと転換していった。本酵素はN－グリコシル化された高マンノース型の糖鎖を有し，前駆体はリン酸糖質を含有していた。前駆体をマイクロゾーム分画からCon－A Sepharoseカラムクロマトグラフィーにより精製し，pH 3.0で加温すると成熟型への変換が認められるとともに，36時間後に活性が210倍に上昇した。この試験管内における変換はペプスタチンによって阻害されることが判明された。この結果はカテプシンLの前駆体への転換にアスパラティックプロテアーゼが関与している可能性を示している。

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